REMARKS/ARGUMENTS

Status of the Claims

Claims 12-19 were rejected. Claims 1-11 were previously cancelled without prejudice or disclaimer. Applicants reserve the right to pursue these claims in a continuation or divisional application. Claims 12-19 are pending in the present application.

The Objection to the Drawings Has Been Withdrawn

Applicants acknowledge the Examiner's acceptance of the formal drawings submitted on April 15, 2004.

The Rejection of the Claims Under 35 U.S.C. § 103 Should Be Withdrawn

Claims 12-16 and 18 were rejected under 35 U.S.C. § 103 as being unpatentable over Woghiren *et al.* (1993) *Bioconj. Chem.* 4:314-318 in view of Miles *et al.* (1997) *Art. Cells, Blood Subs., Immob. Biotech.* 25:315-326, Iwashita *et al.* (1988) *Biomat., Art. Cells, Art. Org.* 16:271-80, Rausch *et al.* (U.S. Pat. No. 5,084,558), Katsunuma (U.S. Pat. No. 4,229,571), and JP 53038617. This rejection is respectfully traversed.

A *prima facie* case of obviousness requires some suggestion to combine the cited references to arrive at the claimed invention and a reasonable expectation of success in such a combination. The claimed invention in the instant case is a method of preparing a chemically modified hemoglobin solution that is substantially free of contaminants comprising dissolving an aPEG in a solvent in which the aPEG is stabile, filtering the aPEG solution to substantially reduce the level of contaminants, and combining the filtered aPEG solution with a hemoglobin solution. Significant reductions in contaminants present in the chemically modified hemoglobin solution result from using a filtered aPEG solution. See, for example, pages 15-17, Example 4 and Tables 2-3. The Examiner asserts that the motivation to combine the cited references to arrive at the claimed methods arises from the desirability of producing a safer, non-toxic hemoglobin solution for use as a therapeutic. This reasoning is insufficient to establish a motivation to combine the references. Moreover, the references, even if combined, would not allow one of skill in the art to produce the claimed invention.

Woghiren *et al.* teach a thiol-protected activated PEG (aPEG) and a method of using it for the modification of cysteine-containing proteins, particularly in cases where derivatization through a lysine is undesirable. Specifically, monomethoxypolyethylene glycol (mPEG) is transformed to a thiol-protected aPEG (i.e., PEG-SS-4TP) through a series of synthetic steps, and the resulting PEG-SS-4TP is used to modify papain. Woghiren *et al.* do not teach or suggest chemically modifying hemoglobin.

The production of PEG-SS-4TP requires several intermediate reactions, such as the preparation of tosyl-PEG, the preparation of PEG-thioacetate, alcoholysis of PEG-thioacetate, and protection of the free thiol, in order to produce the final desired aPEG (i.e., PEG-SS-4TP) that is used to chemically modify papain. At each of these steps, the reaction products are separated from the residual chemical reagents by filtration through Sephadex G-25. The reference does not teach or suggest that this filtration step is intended to or actually does substantially reduce the level of contaminants. In fact, Woghiren *et al.* expressly state that the reaction products are only "partially purified" by Sephadex G-25, indicating that the filtration step does not substantially reduce the level of contaminants, as required by the present invention. See, for example, page 315, right column. Furthermore, in the final synthetic step, the PEG-SS-4TP is isolated by gel filtration as before, frozen, and evaporated to dryness using a vacuum concentrator. The dried PEG-SS-4TP is then resuspended in aqueous MOPS buffer, and the papain protein solution is added drop-wise to a 15-fold molar excess of PEG-SS-4TP. The resulting PEG-papain solution is purified by HPLC and high-performance gel filtration chromatography.

Contrary to the Examiner's assertions, the final aPEG solution (i.e., PEG-SS-4TP resuspended in MOPS buffer) is <u>not</u> filtered or subjected to any purification prior to using it to modify the protein solution. The claimed methods, however, expressly state that the aPEG solution is filtered to substantially reduce contaminant levels and then the resulting <u>filtered</u> solution is combined with a hemoglobin solution. While contaminants such as endotoxin can be removed after PEGylation, purification following chemical modification of the hemoglobin solution results in undesirable changes to the protein composition (e.g., removal of antioxidant enzymes associated with the PHP complex) or even in destruction of the product (page 3, lines 1-3). Therefore, the use of a filtered aPEG solution is critical to the production of a final

hemoglobin product that is substantially free of contaminants. Thus, Woghiren *et al.* do not teach or even suggest an important step in the claimed methods.

The Examiner further asserts that it is implicit from the teachings of Woghiren *et al.* that purification through Sephadex G-25 resin renders the aPEG solution substantially free of contaminants. Again, as discussed above, the final PEG-SS-4TP/MOPS buffer solution that is used to modify papain is not filtered, as required by the claims. Moreover, the purification steps used during the intermediate reactions were designed only to separate the desired reaction products from excess unreacted reagents (e.g., "the excess tosyl chloride was conveniently eliminated from the product in the gel filtration purification step," page 316, left column). Specifically, the G-25 size exclusion chromatography (SEC) employed by Woghiren *et al.* is merely a "desalting" step whereby low molecular weight chemicals (i.e., the reactants) are removed from the aPEG intermediate reaction products. In contrast, the methods of the claimed invention require that the aPEG solution is filtered to substantially reduce the level of contaminants. Thus, the G-25 filtration techniques taught by Woghiren *et al.* are not equivalent to the filtration step required by the present claims.

The term "contaminants," as used in the present application, "refers to compounds including, but not limited to, bioburden, endotoxin, and particulates" (page 6, lines 7-8). Moreover, as defined in the present specification, an aPEG solution that has been substantially reduced in contaminants is "noninfectious" and is "characterized by not inducing pathophysiological effects. . .upon *in vivo* administration to a subject" (page 7, lines 30-31 and page 8, line 5). While the Applicants acknowledge that the SEC separation used by Woghiren *et al.* is effective for the removal of residual reactants during the synthesis of the aPEG, there is no indication or suggestion in the reference that the limited purification steps used would render even the intermediate reaction products substantially reduced in contaminants, as defined in the application. The Examiner further maintains that the final aPEG solution of Woghiren *et al.* was in fact purified because the "PEG-SS-4TP 'gave a single peak' when analyzed by LDMS mass spectrometry" (page 5-6, Office Action mailed July 28, 2004). Contrary to the Examiner's assertions, however, the analytical method used to demonstrate the purity of the aPEG solution in the cited reference would not detect either bioburden or endotoxin contaminants because both possess molecular masses that are too large to be analyzed by LDMS. Thus, the 'single peak' of

Figure 2A does not conclusively demonstrate that the PEG-SS-4TP solution is substantially reduced in contaminants. Furthermore, the final aPEG solution of the cited reference is simply not purified through a filter of any type prior to combining it with the protein solution, as required by the claims.

The Examiner also maintains that Woghiren *et al.* do not need to teach filtering the aPEG solution to substantially reduce the level of bioburden and endotoxin contaminants because "such limitations are not present in the instant claims" (page 6, Office Action mailed July 28, 2004). The Examiner's attention is drawn to claim 15, and claims dependent thereon (i.e., claims 16-19), which do expressly require that the aPEG solution is filtered to substantially reduce endotoxin contaminant levels. Again, the PEG-SS-4TP/MOPS buffer solution of Woghiren *et al.* is not filtered, and, moreover, there is no discussion or suggestion that any of the purification steps would be sufficient to substantially reduce endotoxin levels in even the intermediate reaction products. In fact, the Sephadex G-25 gel filtration taught by Woghiren *et al.* does not permit the removal of endotoxin and bioburden contaminants. The fractionation range of coarse G-25 resin is 100-5000 Da for linear molecules, such as dextrans, and 1000-5000 Da for globular proteins. Endotoxin has an approximate molecular weight of 10,000 Da and often exists as a higher molecular weight aggregate. Thus, G-25 gel filtration chromatography simply cannot be used to substantially reduce endotoxin and bioburden levels because such contaminants co-elute with the aPEG. And finally, Woghiren *et al.* do not suggest that PEG-SS-4TP could be successfully used to modify hemoglobin.

Katsunuma discloses compositions and methods directed to a glucocorticoid sparing factor (GSF). JP 53038617 teaches a method for preparing an inactivated hepatitis B vaccine. Both references generally teach the use of gel filtration and column chromatography for the purification and separation of compositions of interest. Neither reference, however, is directed to hemoglobin solutions or suggests filtering an aPEG solution. Furthermore, the mere fact that these references generally disclose purification techniques does not indicate that a person skilled in the art would have been motivated to filter an aPEG solution to substantially reduce the level of contaminants and then to use the filtered solution to chemically modify hemoglobin, as required by the present invention.

Iwashita *et al.* teach compositions and methods directed to a pyridoxalated hemoglobin-POE conjugate. The modified hemoglobin of Iwashita *et al.* is purified by repeated ultrafiltrations of the final product. Rausch *et al.* disclose a cross-linked, substantially endotoxin-free hemoglobin solution and a method for producing the same. The methods of Rausch *et al.* comprise several filtrations of the unmodified hemoglobin solution in order to remove endotoxin contaminants prior to cross-linking with glutaraldehyde. Neither of these references, however, teaches nor suggests using a stable, filtered aPEG solution to modify hemoglobin.

Miles *et al.* teach an HPLC-based method for the quantitation of residual α-carboxymethyl, ω-carboxymethoxy polyoxyethylene (POE) in chemically modified hemoglobin solutions. The cited reference also acknowledges that the removal of residual chemical modification reagents (e.g., POE) is crucial to the production of a safe product. The Examiner is correct that Miles *et al.* further teach that a need exists for purified hemoglobin solutions for use as therapeutics and that chemical modification of proteins with PEG prevents renal toxicity, increases circulating half-life, and decreases immunogenicity *in vivo*. These observations were known generally in the art. The reference, however, does not teach or suggest that the desired safer hemoglobin products could be obtained by dissolving an aPEG in a solvent in which it is stable, filtering the aPEG solution, and then using the filtered aPEG solution to modify hemoglobin, as required by the claims.

The Examiner asserts that in light of the need for purified hemoglobin solutions recognized by Miles *et al.* and others that one skilled in the art would have been motivated to combine the method of Woghiren *et al.* with the hemoglobin solutions of Iwashita *et al.* or Rausch *et al.* and the disclosure of general gel filtration techniques of Katsunuma and JP 53038617 to arrive at the claimed methods. The mere fact, however, that a need for a safer hemoglobin product was recognized in the art is no indication that one would have been motivated to combine the cited references to produce the desired hemoglobin solution by the methods of the present invention. Moreover, none of the cited references teach or suggest dissolving an aPEG in a solvent, filtering the aPEG solution to substantially reduce the level of contaminants, and then using the filtered aPEG solution to chemically modify hemoglobin.

Therefore, there is insufficient motivation to combine the cited references to arrive at the claimed methods, and, as such, a *prima facie* case of obviousness has not been established.

Furthermore, although there is insufficient motivation to combine the references, even if combined, the references would not allow one of skill in the art to arrive at the claimed invention. As discussed above, Iwashita *et al.*, Rausch *et al.*, Katsunuma, and Miles *et al.* do not teach or suggest dissolving an aPEG in a solvent, filtering the aPEG solution to substantially reduce contaminants, and combining the filtered aPEG solution with hemoglobin, as required by the present claims. Although Woghiren *et al.* do teach the partial purification of intermediate reaction products during the synthesis of the PEG-SS-4TP, the final aPEG solution is not filtered prior to combining it with the protein solution, and, moreover, there is no suggestion in the cited reference that the PEG-SS-4TP could be used to modify hemoglobin. In contrast, the claimed methods recite that the aPEG solution is filtered to substantially reduce contaminant levels and then the resulting filtered aPEG solution is combined with hemoglobin. Filtering the aPEG solution and using the filtered aPEG solution to modify hemoglobin are important steps in the present methods for producing a hemoglobin solution that is substantially free of contaminants. Moreover, *prior to the present disclosure it was not known that a stable aPEG solution could be produced, filtered to substantially reduce contaminant levels, and successfully used to modify a hemoglobin solution.* Accordingly, as none of the references teach or suggest the critical steps of filtering an aPEG solution to substantially reduce contaminant levels and combining the filtered solution with a hemoglobin solution, the combination of references could not have placed the invention of claims 12-16 and 18 in the hands of the public, and a *prima facie* case of obviousness under 35 U.S.C. § 103 has not been established.

The Examiner further concludes that claims 17 and 19 are obvious in view of the above references as modified by Feola *et al.* (U.S. Pat. No. 5,439,882). Dependent claims 17 and 19 further comprise filtering the aPEG solution through a 0.2 micron nylon filter. Feola *et al.* teach the use of a 0.2 micron Posidyne® filter to remove contaminants from an extracted, unmodified hemoglobin solution. The Examiner maintains that Feola *et al.* provide evidence that "the use of a Posidyne® 0.2 micron filter to further remove microbial contaminants from hemoglobin preparations was routine and conventional in the art" (page 7, Office Action mailed July 28, 2004). Therefore, the Examiner concludes that it would have been obvious to one of skill in the

art to use the filter disclosed by Feola *et al.* in conjunction with the method of Woghiren *et al.* and the disclosures of Miles *et al.*, Iwashita *et al.*, or Rausch *et al.* to arrive at the methods of claim 17 and 19.

In contrast to claims 17 and 19, Feola *et al.* disclose only the use of a 0.2 micron filter to remove contaminants from an extracted hemoglobin solution. Feola *et al.* do not teach or suggest using an aPEG to chemically modify a hemoglobin solution, and, therefore, also do not suggest using a filter of any size to remove contaminants from an aPEG solution. Even if, as the Examiner suggests, the use of a 0.2 micron filter to remove contaminants from a hemoglobin solution was routine, Feola *et al.* provide no evidence that an aPEG could be dissolved in a solvent and filtered through a 0.2 micron filter, or any type of filter, to substantially reduce contaminants. Moreover, as noted above, none of the cited references teach or suggest filtering an aPEG solution to substantially reduce contaminant levels prior to using the filtered aPEG solution to chemically modify hemoglobin, as required by the claimed methods. Furthermore, claim 19 contains the additional limitation that the steps of filtering the aPEG solution and combining the filtered aPEG solution with the hemoglobin solution are aseptically joined. This requirement for aseptic coupling of the filtering and combining steps is not suggested in any of the references cited by the Examiner. Therefore, claims 17 and 19 are not obvious in view of the cited references.

For the reasons presented above, the Examiner has failed to establish a *prima facie* case of obviousness. Accordingly, Applicants respectfully submit that the claimed methods for producing a chemically modified hemoglobin solution that is substantially free of contaminants are not obvious in view of the cited references and request that the rejection of claims 12-19 under 35 U.S.C. § 103(a) be withdrawn.

CONCLUSION

The Examiner is respectfully requested to withdraw the rejections and allow claims 12-19. In any event, the Examiner is respectfully requested to consider the above remarks submitted herewith for the purposes of further prosecution.

Accordingly, in view of the above remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited.

Appl. No.: 09/934,300
Amdt. dated 10/26/2004
Reply to Office action of July 28, 2004

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned.

Consideration Of Previously Submitted Information Disclosure Statement

It is noted that an initialed copy of the PTO Form 1449 that was submitted with Applicants' Information Disclosure Statement filed September 17, 2004 has not been returned to Applicants' representative with the Office Action. Accordingly, it is requested that an initialed copy of the Form 1449 be forwarded to the undersigned with the next communication from the PTO. In order to facilitate review of the references by the Examiner, a copy of the Information Disclosure Statement and the Form 1449 are attached hereto. Copies of the cited references were provided at the time of filing the original Information Disclosure Statement, and, therefore, no additional copies of the references are submitted herewith. Applicants will be pleased to provide additional copies of the references upon the Examiner's request if it proves difficult to locate the original references.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

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CERTIFICATION OF FACSIMILE TRANSMISSION

I hereby certify that this paper is being facsimile transmitted to the U.S. Patent and Trademark Office Fax No. (703) 872- 9306 on October 26, 2004.

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